Mutations in *INPP5K* cause a form of congenital muscular dystrophy syndrome overlapping Marinesco-Sjögren Syndrome and the dystroglycanopathies

Short title: *INPP5K* mutations cause CMD

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Abstract

Congenital muscular dystrophies display a wide phenotypic and genetic heterogeneity. The combination of clinical, biochemical and molecular genetic findings must be considered for obtaining the precise diagnosis and providing appropriate genetic counselling. Here we report five individuals from four families presenting with variable clinical features including muscular dystrophy with a reduction in dystroglycan glycosylation, short stature, intellectual disability and cataracts, overlapping both the dystroglycanopathies and Marinesco-Sjögren syndrome. Whole-exome sequencing revealed homozygous missense, and compound heterozygous mutations in *INPP5K* in the affected members of each family. *INPP5K* encodes the inositol polyphosphate-5-phosphatase K, also known as *SKIP* (Skeletal muscle and Kidney enriched Inositol Phosphatase), which is highly expressed in the brain and muscle. INPP5K localizes to both the endoplasmic reticulum and to actin ruffles in the cytoplasm. It has been shown to regulate myoblast differentiation and has also been implicated in protein processing through its interaction with the ER chaperone HSPA5/BiP. We show that morpholino-mediated *inpp5k* loss of function in the zebrafish results in shortened body axis, microphthalmia with disorganized lens, microcephaly, reduced touch-evoked motility and highly disorganized myofibers. Altogether these data demonstrate that mutations in *INPP5K* cause a congenital muscular dystrophy syndrome with short stature, cataracts and intellectual disability.

Main text

Congenital muscular dystrophy (CMD) encompasses a group of disorders characterized by muscle weakness and progressive loss of muscle mass and function, presenting at birth or infancy[1,2]. Multiple forms of CMD are also associated with cerebral and ocular phenotypes, suggesting common mechanisms affecting development of the muscle, brain

and eye. For these syndromic forms, gene identification studies have primarily pointed to a molecular mechanism involving interactions between cells and the surrounding extracellular matrix (ECM)[3]. Mutations in up to nineteen glycosyltransferases and accessory proteins involved in the glycosylation of the transmembrane glycoprotein dystroglycan (*DAG1* [MIM:128239]) lead to a spectrum of CMDs termed dystroglycanopathies[4,5] . Dystroglycanopathy-associated genes function in the endoplasmic reticulum (ER) and/or Golgi apparatus to regulate dystroglycan glycosylation. Genetic and function studies have shown that glycans are critical to control normal tissue development in the brain, eye and muscle, via interactions with the ECM[5]. The most severe forms of dystroglycanopathy present with CMD associated with lissencephaly (smooth brain) and a variety of eye malformations affecting both the retina and the anterior chamber (e.g. cataracts, glaucoma), but multiple cases have only CMD with intellectual disability and more subtle brain findings[6,7].

Marinesco-Sjoegren syndrome (MSS, [MIM:248800]) is a form of myopathy with a similar constellation of findings including muscle involvement, intellectual disability, cataracts, brain MRI findings, and other signs of central nervous system (CNS) involvement[8,9]. Cerebellar atrophy is often considered the most prominent neuroradiologic finding in MSS, however it is not an obligatory finding[10]. The clinical overlap can therefore make it difficult to distinguish between syndromic CMDs and MSS. MSS is also considered to be a clinically and genetically heterogeneous disorder with approximately 70% of MSS cases harboring mutations in the SIL1 Nucleotide Exchange Factor (*SIL1* [MIM:608005])[8]. SIL1 acts as a nucleotide exchange factor for Heat Shock Protein family A member 5 (HSPA5), also known as GRP78 (Glucose-related protein 78) or BiP (immunoglobulin binding protein), an essential regulator of ER function, and has led to the suggestion that MSS is a disorder of protein biosynthesis or processing in the ER[11].

In this report, we present five individuals from four families diagnosed with a syndrome overlapping both the dystroglycanopathy and the MSS spectrum with recessive mutations in Inositol Polyphosphate-5-Phosphatase K (*INPP5K* [MIM:607875]) **(Figure 1)**. INPP5K belongs to a family of phosphatidylinositol (PI) phosphatases responsible for removing the phosphate on position 5 of the inositol ring leading to $PI(3,4)P_2$ from $PI(3,4,5)P_3$ and PI4P from $PI(4,5)P_2$. Also known as Skeletal muscle and Kidney-enriched Inositol Phosphatase *(SKIP), INPP5K* is highly expressed in the developing and adult brain, eye and muscle[12-14]. It is primarily localized to the ER, and it can form a complex with HSPA5/BiP to regulate insulin receptor signaling at actin ruffles on the plasma membrane by acting as a negative regulator of phosphatidylinositol-3-kinase (PI3K) signaling[15], suggesting a possible overlap with the function of the MSS-associated gene *SIL1*.

In agreement with the Declaration of Helsinki, informed consent for genetic and biochemical studies was obtained from all study participants or their guardians under the authority of the George Washington University Internal Review Board and the Narges Medical Genetics and Prenatal Diagnosis Laboratory. Subject 1.1 and 1.2 (S1.1 and S1.2, **Figure 1A, Table 1**) are two affected sisters (7- and 13-year old), born of an Arabian consanguineous family in Iran who were initially diagnosed with muscle atrophy and global developmental delay. Both were born without complications, and early development was normal with the exception of delayed walking from 18 months following a period of occupational therapy. Both sisters experienced febrile seizures in infancy (9 months) and early childhood (2 years), and routine interictal electroencephalography in the elder sister was normal. They displayed difficulty rising from a squatting position, impaired toe-standing and unsteady gait. At the age of 2 the elder sister began to show hypotonicity which progressed to spasticity including diffuse loss of muscle bulk, exaggerated deep tendon reflexes, positive pyramidal signs and fatty infiltration resulting in frequent falls, toe

walking, spastic gait and pronounced hyperlordosis. Both sisters cannot climb stairs without support. Following orthopedic surgery the eldest sister uses a wheelchair and muscle biopsy showed myogenic atrophy. Electromyogram (EMG) report also showed the presence of myopathy involving both her lower limbs, with more involvement of the proximal muscles. Both sisters have increased serum creatine kinase (CK), aldolase and alkaline phosphatase levels. They have moderate to severe intellectual disability (ID), both attending special schools. Brain magnetic resonance imaging (MRI) of S1.1 was normal at the age 8 and 13. Both sisters have short stature, microcephaly, and impaired speech. Variable clinical features include bilateral cataracts in S1.2.

Whole exome sequencing (see **Table S1** for details) was performed on S1.1 focusing on the identification of potentially deleterious rare homozygous variants due to the presence of multiple large regions of homozygosity. Thirteen candidate genes were identified with homozygous missense mutations (**Table S2**). A homozygous variant in *INPP5K* (NM_016532, **Table S3**) was identified within an 8Mb region of homozygosity (c.277A>G; p.Met93Val) (**Figure 1A-B**). Sanger sequencing confirmed that both affected children were homozygous for this variant and each parent was heterozygous for the variant. The variant is unique in an in-house dataset of 450 geographically matched individuals sequenced by exome, and is not represented in the Greater Middle Eastern Variome[16] , in 60,706 individuals in the Exome Aggregation Consortium (ExAC) Browser[17], or in 141,353 individuals in the Genome Aggregation Database (gnomAD). It is highly conserved (**Figure 1C**) and predicted to be pathogenic by multiple prediction tools (SIFT=0.01; CADD=22.7)[18] (**Table S3**).

Subjects 2 (S2) and 3 (S3) were simplex cases originating from Southern Italy. S2 (**Figure 1A, Table 1**) is 31 years old and was referred with a diagnosis of CMD as a child. She was born at term with low birth weight and presented with motor and cognitive delay

since early infancy. At 2 years of age she underwent surgery for bilateral cataracts. Upon neuromuscular examination at age 5, she was able to walk independently, but her upper limbs were hypotonic and hypotrophic with no tendon reflexes, and lower limbs were slightly hypertonic with brisk tendon reflexes. CK and aldolase levels were reported as higher than normal. EMG revealed myopathic changes with normal sensory and motor conduction velocities. A biopsy of the quadriceps muscle showed neuromyogenic changes. Fiber diameter was variable, with several small rounded or wedge-shaped fibers, mildly increased perymisial connective tissue, rare centrally located nuclei, and a few degenerating and regenerating fibers (H&E staining, **Figure 2A**). α-dystroglycan immunohistochemistry using either the VIA4-1 or IIH6 antibody (Millipore) showed reduced protein glycosylation on fiber surfaces (**Figure 2C**). Dystrophin-associated glycoproteins and laminin-α2 were normally expressed (not shown). In addition to the neuromuscular phenotype, she presented with dysmorphic features: short stature, thin hair, globular nose and micrognathism, plus skeletal anomalies such as 13 ribs and scoliosis. A cerebral CT showed an elongated (dolichocephalic) skull, with underdevelopment of both cortex and white matter of cerebral hemispheres. She was treated for focal seizures until she was 10 years-old. A brain MRI, performed at age 18 years, showed an anomaly of the craniovertebral junction (odontoid process in the foramen magnum), and enlarged lateral ventricles and subarachnoid spaces, suggesting progressive brain atrophy. During follow-ups, the subject's clinical features remained stable. She presents with moderate-severe ID without speech deficits and is very sociable. At 22 years of age she could still walk independently, but needed handrail support when going up and down stairs. Strength evaluation showed moderate proximal weakness of upper limbs, and moderate-severe proximal and distal weakness of lower limbs with initial retractions of adductor muscles and Achilles tendons. Due to a fall resulting in a femur fracture at age 28, she is now wheelchair-bound.

S3 (**Figure 1A, Table 1**) is a 21-year-old male who also presented with delayed psychomotor development at 23 months following an uneventful pregnancy and delivery. Neurological examination at 3 years of age identified marked axial and limb hypotonia with proximal upper and lower limb weakness, marked lumbar hyperlordosis and areflexia. Gower's sign was positive. CK was elevated often above 1,000U/L and muscle biopsy showed neuromyogenic changes. A mild increase of perimysial connective tissue was noted with variable fiber size with hyper-, hypo- and atrophic fibers, a few centrally located nuclei, split fibers, rare degenerating fibers and small type groupings (Gomori trichrome staining, **Figure 2B**). α-dystroglycan immunohistochemistry showed reduced glycosylation expression on fiber surfaces (**Figure 2C**), while dystrophin, dystrophin-associated glycoproteins and laminin-α2 were normally expressed. He was moderately dysmorphic with an elongated face. Mild ID and microcephaly were noted, but brain MRI was normal. During subsequent years muscle weakness slowly worsened with loss of autonomous gait at age 12. No cardiac impairment was noticed at echocardiographic examinations, while a mild restrictive respiratory deficiency was noticed since adolescence. No eye involvement was reported until the last visit at age 21.

S2 and S3 underwent exome sequencing as part of a cohort of Italian cases of CMD with variable brain abnormalities and cognitive deficits (see **Table S1** for details on exome). Exomes were filtered using custom SQL queries for rare (<0.5% frequency in the ExAC Browser for both total and non-Finnish European populations) and likely pathogenic (missense, splicing or truncating) variants [19]. Remaining variants were visually analyzed on the Integrative Genomics Viewer (IGV)[20], leading to three candidate genes in S2 and three candidates in S3 (**Table S2**). Both individuals had biallelic missense variants in *INPP5K* (**Figure 1A-B**). S2 carries compound heterozygous transitions c.67G>A (p.Val23Met) and c.805G>A (p.Asp269Asn), while S3 is homozygous for c.67G>A (**Table S3**). Both variants are

conserved (**Figure 1C**), found in less than 1:100,000 alleles in the ExAC and gnomAD browsers and are predicted to be pathogenic (p.Val23Met: SIFT=0; Polyphen2=1; CADD=16.7. p.Asp269Asn: SIFT=0; Polyphen2=1; CADD=34) (**Table S3**).

Finally, Subject 4 (S4, **Figure 1A, Table 1**) originated from the United States and was referred to us via GeneMatcher[21,22] following clinical exome sequencing in the trio (proband and parents). She is a 17-year-old female who presented in early childhood with delay in reaching motor and cognitive developmental milestones. She walked independently at 18 months. She did not babble until age 3 and had phrases at age 4. Bilateral cataracts were identified at 2 years of age and surgically corrected. Since childhood she had upper and lower limb hypotonia, leading to balance problems and frequent falls. CK is elevated above 1,000. Currently, she has an appreciable loss of muscle mass in her hands and feet. She toe walks and fatigues easily. EMG at 16 years of age was normal. A muscle biopsy has not been performed. She has short stature for which she was treated with growth hormone. Other findings include hirsutism, microcephaly and moderate ID. Brain MRI at age 16 was normal. Clinical exome sequencing was performed by GeneDX (Gaithersburgh, MD) using proprietary capture chemistry (**Table S1** for details). Analysis identified *INPP5K* as the most likely mutated gene. S4 carries a likely pathogenic transition and a small deletion in compound heterozygosity, c.418G>A (p.Gly140Ser; SIFT=0; Polyphen2=1; CADD=28.6) and c.1251_1252delCA (p.Asn417Lysfs*26) (**Figure 1A-B**). The missense variant is conserved and extremely rare, and the deletion is not found in ExAC and gnomAD browsers (**Table S3**).

In summary, all individuals with likely pathogenic biallelic mutations in *INPP5K* present with myopathic findings and elevated CK, short stature, motor and cognitive developmental delay since early infancy and moderate-severe ID. Cataracts were present in S1.1, S2 and S4, but not S1.1's sibling S1.2 showing variability even within the same family.

While no ataxia or cerebellar atrophy was noted, this presentation is reminiscent of MSS and could fall in the larger MSS spectrum[23]. In addition, a reduction in dystroglycan glycosylation in S2 and 3 suggest an overlap with less severe forms of dystroglycanopathies, where CMD is present with cataracts and ID[6,7]. Similar cases of merosin-positive CMD with cataracts and ID have been previously reported[24,25], also suggesting that this disorder may represent a distinct clinical entity.

Since the identified variants are either missense or late truncations, we sought to determine their impact on protein function by performing phosphatase activity assays. GST-tagged full-length *INPP5K* constructs (wild type and mutants) were assayed for their activity against 135mM PtdIns(4,5)P_2diC8 soluble lipid substrate in phosphatase assay buffer (50 mM Tris-HCl, pH7.5, 150 mM NaCl, 10 mM $MgCl_2$). Free phosphate was measured using the Malachite Green assay kit (Echelon Biosciences, Salt Lake City, UT) and calibrated against standards according to the manufacturer's instructions. To minimize variability between purifications, all constructs were freshly prepared and purified in parallel for each experiment, and beads used in the assay were afterwards run on Coomassie gels to confirm equal protein loading. We found that when compared to a phosphatase-dead construct (p.Asp310Gly), p.Gly140Ser and p.Asp269Asn had almost no enzymatic activity, while p.Val23Met and p.Met93Val retained 27% and 42% activity, respectively. The C-terminal frameshift deletion p.Asn417LysfsX26, which is located outside of the phosphatase domain, was the least severely disrupted (57% activity in this assay) (**Figure 1D**).Both S1.1/2 and S3 are homozygous for variants that only partially reduce phosphatase activity of INPP5K, but analysis of more cases will be necessary to establish genotype/phenotype correlation.

To explore the role of *INPP5K* during early development we targeted *inpp5k* in the zebrafish embryo using antisense morpholino oligonucleotides (MOs, GeneTools, LLC). Teleost fish underwent a genome duplication event, leading to approximately 30% of genes

having a paralogue[26]. *inpp5k* is present in two copies in the zebrafish genome: *inpp5ka* and *inpp5kb*. Alignment of both homologues indicate *inpp5ka* is more similar to *INPP5K* (**Figure S1**), suggesting it is the closest orthologue of the human gene. In addition, quantitative PCR expression analysis in the zebrafish embryo indicated that *inpp5ka* expression is, respectively, 7-fold and 16-fold higher than *inpp5kb* at 1 and 2 days post fertilization (dpf) (**Figure S1B**). MOs were designed by two independent groups to target both the start site (translation-blocking) and splicing of the *inpp5ka* and *b* mRNA, leading to four independent MOs being tested for each gene (**Table S4, Figure 3, Figure S2-S4**). Experiments were also performed on two independent strains, TupLF and AB.

Injections of *inpp5ka* MOs in the fertilized oocyte resulted in a striking phenotype in zebrafish embryos and larvae, which featured microphthalmia, microcephaly, curved and shortened body and reduced touch evoked motility (**Figure 3A, Figure S2-S4, Supplemental video 1-5**). This phenotype was consistent across all *inpp5ka* MOs injections and in the *inpp5ka* and *b* double morphant, while *inpp5kb* MOs alone showed very mild phenotypes (**Figure S2A-D and S4**). It is not uncommon for one gene in a pair of duplicated orthologues to lose its function or be silenced[27]. Therefore, all subsequent analysis was performed on *inpp5ka* morphants to reduce as much as possible the amount of MO injected in the embryos and avoid non-specific effects. *inpp5ka* morphants phenotypes could be significantly improved by co-injection of 200pg of capped human *INPP5K* mRNA, while injection of *INPP5K* mRNA alone had no effect (**Figure S2E-F**).

Examination of the eye by wax sectioning followed by H&E staining showed that in morphants eyes are orientated downwards, as indicated by the position of the lens (**Figure 3B**). At 3 dpf the lens organizes in a cellular cortex and an acellular nucleus[28]. α-crystallin (zl-1; Zebrafish International Resource Center) immunostaining in axial sections of the *inpp5ka* morphant showed that the lens cortex was disorganized and cell nuclei were

present in the center of the lens nucleus leading to a phenotype reminiscent of congenital cataracts (**Figure 3C**).

A birefringence assay using polarized light to image the densely packed and highly organized nature of muscle fibers of control embryos showed that knockdown of *inpp5ka* caused a substantial reduction in birefringence (**Figure 3D**), suggesting muscle fiber disorganization. To better evaluate skeletal muscle structure, phalloidin (Molecular Probes) was used to mark filamentous actin (F-actin) in sarcomeres. Control embryos display densely packed, organized muscle fibers in the trunk of the zebrafish embryo (**Figure 3E**). *inpp5ka* morphants showed sparser, disorganized myofibers, with the appearance of 'wavy fibers' (**Figure 3E**). As the role of INPP5K has not been previously characterized we investigated its potential role in the formation of neuromuscular junctions (NMJs). Analysis of NMJs, by targeting the acetylcholine receptors (AChR) using the high affinity fluorophore conjugated alpha-Bungarotoxin (Molecular Probes), reveals reduced synaptic formation in the skeletal muscle of *inpp5ka* knockdown embryos (**Figure 3E**). Thus, *inpp5ka* is required for appropriate formation of skeletal muscles and NMJs.

Finally, transmission electron microscopy (TEM) was used to expose sarcomeric assembly defects consistent with reduced motility in the morphants (**Figure 4A**). Whilst control embryos display clearly defined electron dense anisotropic (A-bands) and less dense isotropic (I-bands) bands, the morphants have undefined A- and I- bands (**Figure 4B**). Sarcomere length is shorter in *inpp5ka* knockdown embryos, compared to control MO injected or uninjected embryos, a phenotype normally associated with contracted sarcomeres. In addition, myofibrils are loosely packed with a disorganized arrangement in morphants. Triads, consisting of T-tubules and sarcoplasmic reticulum (SR), are required for sarcomeric contraction. Knockdown of *inpp5ka* results in triads which are on average half the size of those in control embryos (**Figure 4C-E**), a possible feature of an exhausted

SR[29]. Muscular dystrophy phenotypes are associated with detachment of myofibres at somite borders[30]. We analyzed muscle fiber attachment at the somite borders in knockdown and control embryos (**Figure 4C**). Control embryos showed well defined attachments to the myoseptum, which were reduced in morphant embryos. Muscle fiber detachments were not observed in the F-actin immunofluorescent staining, suggesting that whilst the myoseptal attachments are present, they may be weaker. Taken together, these data suggest that inpp5ka is required for appropriate sarcomere assembly and function.

In summary, we identified five independent alleles in *INPP5K* leading to a disorder characterized by short stature, ID, CMD and cataracts. *inpp5k* loss of function modeling in the zebrafish led to a constellation of phenotypes that closely resembles the human presentation, including reduced growth, microcephaly, lens disorganization, reduced motility and myopathy. The clinical presentation partially overlaps with MSS, and with at least two subjects showing a reduction in dystroglycan glycosylation suggests a continuum with the dystroglycanopathies. This combination of phenotypes could be explained by both by INPP5K enzymatic function and by its binding partners in the ER. Because of its role in converting PIP_2, INPP5K has been shown to promote myoblast differentiation by controlling the myogenic loop triggered by insulin-growth factor II (IGF-II) through the PI3K-AKT-mTOR pathway[31]. Little is known about the role of INPP5K in the brain, but IGF-II activity is also critical for learning and memory consolidation[32]. It is possible that changes in AKT-mTOR activity in the brain will contribute to cognitive deficits as this signaling pathway has been found disrupted in multiple neurodevelopmental disorders[33]. In addition, INPP5K's signaling activity is directly regulated by its binding partner HSPA5/BiP[15,34], which is in turn regulated by the MSS-associated gene *SIL1*[11]. SIL1 regulates the activation stage of HSPA5/BiP in the ER and BiP is a critical chaperone for trafficking of glycoproteins[11]. Therefore, INPP5K could alter dystroglycan targeting by altering HSPA5/BiP function.

Future studies must address the role of INPP5K in signaling regulation and glycoprotein trafficking during brain and muscle development.

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Web resources

Online Mendelian Inheritance of Man: http://www.omim.org/
Greater Middle Eastern Variome: http://igm.ucsd.edu/gme/index.php
ExAC Browser: http://exac.broadinstitute.org/
gnomAD Browser: http://gnomad.broadinstitute.org/
Polyphen 2: http://genetics.bwh.harvard.edu/pph2/
SIFT: http://sift.jcvi.org/
ANNOVAR: http://www.openbioinformatics.org/annovar/
HomoloGene: https://www.ncbi.nlm.nih.gov/homologene/
Ensembl genome browser: https://www.ensembl.org/
FANTOM5: http://fantom.gsc.riken.jp/5/

Table 1. Clinical features of individuals with *INPP5K* mutations

Subject	S1.1	S1.2	S2	S3	S4
Protein change	hom p.Met93Val	hom p.Met93Val	p.Val23Met/ p.Asp269Asn	hom p.Val23Met	p.Gly140Ser/ p.Asn417Lysfs*26
% WT function	42±9%	42±9%	27±7%/10±8%	27±7%	13±1%/58±10%
Ethnic Origin	Iranian-Arab	Iranian-Arab	Italian	Italian	European American
Sex	F	F	F	M	F
Current Age	13y	7y	31y	21y	17y
Short stature	+	+	+	+	+
Cataracts	-	+	+	-	+
Strabismus	+	+	-	-	+
Spine hyperlordosis	+	+	-	+	-
Muscle weakness	+	+	+	+	+
CK	1041U/L	1184U/L	Elevated	>1000U/L	Elevated
EMG	Myopathic changes	n/a	Myopathic changes	Myopathic changes	n/a
Muscle biopsy	Myogenic atrophy	n/a	Dystrophic Reduced α-DG	Dystrophic Reduced α-DG	n/a
Spasticity	+	+	+/-	-	+
Mobility	Wheelchair-bound	Assisted walking	Wheelchair-bound	Wheelchair-bound	Ambulatory
Intellectual disability	Moderate/Severe	Moderate/Severe	Moderate	Moderate	Moderate
Seizures	+	+	+	-	-
Microcephaly	Borderline	+	-	+	+
MRI	Normal	n/a	Brain atrophy	Normal	Normal

Abbreviations: WT=wild type, y=years, n/a=not assessed, α-DG=α-dystroglycan

Figure legends

Figure 1: Mutations in *INPP5K* severely disrupt protein function. (A) Pedigrees of Families 1 to 4 where five autosomal recessive alleles in *INPP5K* have been identified by exome sequencing. **(B)** Four missense mutations are localized to the phosphatase domain in INPP5K, while a C-terminal frameshift affects the last actin ruffle targeting domain. **(C)** Protein conservation in the four missense INPP5K mutated amino acids. Percent conservation (cons) to the human gene is shown and * indicates conservation across all species listed. **(D)** Phosphatase activity was measured by using Malachite Green dye to detect GST-INPP5K-mediated phosphate release in the presence of the soluble lipid substrate $PI(4,5)P_2$ diC_8, showing that all reported mutations compromise the enzymatic activity of INPP5K. Results of three independent experiments were presented as mean $\pm$ standard deviation. See **Table 1** for numeric values.

Figure 2: Muscular dystrophy and loss of dystroglycan glycosylation in subjects S2 and S3. (A) Haematoxylin and Eosin (H&E) staining from normal control (con) and subject S2 and **(B)** Gomori trichrome staining from control (con) and subjects S3. Both biopsies show great fiber size variability in, and increased perimysial connective tissue and regenerating fibers are indicated by arrows in S2. Scale bar: 100 μm. **(C)** Immunostaining of α-dystroglycan glycosylation in muscle biopsies from S2 and S3, showing reduced and irregular protein expression in the affected individuals compared to the control (con). Scale bar: 50 μm.

Figure 3: Knockdown of *inpp5ka* in zebrafish causes defective eye development and muscle formation. (A) General morphological abnormalities were observed in *inpp5ka* morphant embryos at 4 dpf, compared to control morpholino (MO) or uninjected control

age matched embryos. Scale bar: 500μm. **(B)** Aberrant eye development was observed in *inpp5ka* morphant wax sections stained with H&E, eyes were observed reduced in size and downwardly oriented. Scale bar: 200μm. **(C)** Structural analysis of the lens, by staining for alpha-Crystallin, identifies disorganization in *inpp5ka* morphant embryos. In addition, abnormal medial nuclei retention was observed in *inpp5ka* morphant lens, as marked by DNA stain Hoechst. Scale Bar: 50μm. **(D)** Birefringence of *inpp5ka* morphant embryos reveals decreased muscle integrity compared to uninjected or control MO injected embryos at 4 dpf. Scale bar: 200μm. **(E)** Analysis of muscle fiber and neuromuscular junction formation using Phalloidin (Red, F-actin) and alpha-Bungarotoxin (Green, AChR), respectively, reveals misaligned myofibers and reduced NMJ arborization in morphants. Scale bar: 100μm.

Figure 4: Zebrafish loss of *inpp5ka* results in disorganized myofibrils and insufficient sarcomere assembly, as analyzed using transmission electron microscopy (TEM). (A) Assessment of sarcomere assembly at the ultrastructural level shows less compact myobrils, short sarcomere length, and undefined regional divisions. Scale bar: 2μm. **(B)** High magnification image of sarcomere assembly indicates the loss of defined anisotropic (black bracket) and isotropic (blue bracket) bands in morphant embryos. T-tubules are also notably smaller than control embryos (arrows). Scale bar: 2 μm. **(C)** Muscle fiber attachments at the somite borders presented disorganized and weak unions with the myoseptum in morphants embryos. Scale bar: 1μm. **(D)** Top panel shows a schematic diagram of the Triad assembly, composed of the sarcoplasmic reticulum (SR) and T-tubule (T) sandwiched between muscle fibers (MF). Middle and lower panels show representative triad morphology for control and inpp5ka morphant fish, respectively. **(E)** Triad size was

quantified using the combined area of SR and T per triad. Con 58.2 ± SEM 7.254 n=10, *inpp5ka*MO 28.3 ± SEM 2.371 n=10. ** P≤0.01, as analyzed using an unpaired T-test.

Supplemental Data

Supplemental Data includes four figures and four tables and can be found this article online at URL

Supplemental Videos

Five Supplemental Videos show reduced touch evoked mobility of *inpp5ka* morphants compared to controls at 3dpf. Light touch with a glass pipette elicits an escape response in un-injected (Video 1) and control MO injected larvae (Video 2), while *inpp5ka* MO injected larvae are bent and immobile. MO injections are as follows: 1ng inpp5ka AUG1 MO (Video 3), 2ng inpp5ka AUG1 MO (Video 4), 2ng inpp5ka I3E4 MO (Video 5).

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